Maybe should make BSKS II+ vector of Hind III (blunt) / Hind III to clone in full length 5.2 kb PSE;

∴ do a double digest

CIP

prepare for vector

I should also make an XbaI vector of BS KS II+

p15, 81 a	25 µl BS KS II+ p4-114 ✓	p15, 81 b	25 µl BS KS II + p4.11
	10 µl NEB 2 ✓		10 µl NEB #2 ✓
	55 µl H₂O ✓		10 µl 10X BSA ✓
	5 µl Hind II ✓		50 µl H₂O ✓
	5 µl Hind III ✓ To 37° 3:15 P.M.		5 µl XbaI ✓

I have become interested in designing an Adenovirus (say Ad5) that can only replicate in prostate + PSA cells e.g. LNCaP. If such a virus could be made eg E1A driven by the PSA enhancer we could have something quite marvelous. It would be a tissue-specific virus.

One approach would be to drive E1A w/ PSE, and grow virus first in 293 as a replication competent virus, purify virus and infect LNCaP - get a productive infection. Now virus would only grow in 293 & LNCaP w/ added hormone, R1881; And in no other cell.

The mutant would be made to have a unique restriction site to easily identify amongst plaques, or PCR of minimal PSE sequences.

What is the nature of the E1A enhancer / promoter in WT Ad5? Can it be functionally replaced w/ minimal PSE?

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Witnessed & Understood by me, [signature] Date

Invented by D. Henderson Date

Recorded by D. Henderson

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w/ SV40 polyA

To pull out β-gal cut w/ XbaI. To clean the vector use BspHI, ScaI, XmnI or NaeI, then β-gal runs as the upper single band.
I have not yet tested dropping the vector w/ the enzymes listed above.

If we wanted to make a smaller CAT we could clone it into NotI sites. That drops ~700 bp of SV40 out of CAT. But our CAT is already small enough for Adeno.

Back to β-gal. We should put in the BglII/HindIII piece then the minimal enhancer, then β-gal. Could PCR the ~~Bgl~~ minimal enhancer-promoter to have two cloning steps.

AGENDA

I. SAB Meeting - Post-mortem
   changes in emphasis
   changes in deployment of personnel

II. Construction of Clones - Eric and Dan
   Within XbaI-ApaI - characterize and transfer to CAT construct - Eric and Joe
   Internal deletion mutants within PSE CAT - CN13 and CN42
      NdeI/Bgl II 1861 bp
      StuI/Bgl II 1421 bp — XbaI - 3729 = [illegible]
   Cla I deletion
   Minimal enhancer driving β-gal - Dan

III. Transfection of PSE clones in LNCaP - Eric

IV. Tissue Specificity of Minimal Enhancer — LNCaP - AR mutation

V. Construction of Adenovirus vector — Cotransfection

VI. Animal Tissue Work-up - Gail
   Hand grind (Hand) vs. Tissue-Tearer (TT) — Can we TT if carefully, [illegible] tubes, [illegible] for other tubes
   Protein Concentration
   Protease Inhibitors
   Decision vs PCR and rtPCR

VII. Liposomes - Henry, Gail and Lena
   DOTMA and DOPE liposomes
   Formulation
      Charge-Ratio, Osmolarity, Gel-Electrophoresis,
   Trip to INEX and Peter Cullis - Henry

VIII. Direct Injection of DNA into Tumors - Eric
   Technique of Intra-tumor Injection
   Assay by CAT, β-gal, histology?
   Histology Lab source in Concord
   Help from Dermatology

Liz in dermatology
F. Sypke - short blonde hair

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(cont.)

I also continued the thoughts on a prostate-specific replication competent Ad5. Adeno 5 is not oncogenic, infects essentially all cell-types. If I recall correctly, Adeno binds to an ICAM as its cell surface receptor.

The notion continues to replace the promoter/enhancer of E1A w/ the minimal PSE. Then to grow in 293 or LNCaP to get replication competent virus.

Today's issue is biological containment. WE Adeno 5 is more dangerous ~~than~~ than a prostate-specific Adeno. Indeed we are narrowing the host range to prostate cells. The dose should be important as well as the route of delivery. While that containing and disability of Adeno may be enough containment it is also easy enough to put in HSV-tk gene driven by either the minimal PSE enhancer, or enhancer/promoter responsive to E1A binding.

Now the virus could be shut down by acyclovir. e.g. it is positively contained. This would probably fly for IV injection.

Does the Adeno 5 DNA polymerase incorporate the defective ~~thymidine~~ guanosine triphosphate made from acyclovir & HSV-tk? unknown

I talked w/ Jonathan Simons today & he has data that says: HSV-tk and acyclovir does not inhibit Adeno - at least the plate was wiped out. - But would it have been wiped out anyway? Isn't that what HSV-tk will do if infection is going on. What would we expect w/ HSV-tk in ~~an~~ a replication defective Adeno

what would wipe out virus [illegible]

- search on Adeno DNA polymerase - ask Joe Nevins
has anyone purified the enzyme

how about cytosine arabinoside

I talked w/ Jonathan about this idea & he liked it. I hope he doesn't spread the idea. I think it promising & somewhat revolutionary - an ~~the~~ engineered tissue-specific virus for therapy.

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